Cortical development: **Layers of complexity**

Edward C. Gilmore* and Karl Herrup†

Studies of spontaneous mutant mice with neurological phenotypes, particularly the cloning and analysis of the genes responsible, are shedding light on the complex processes that lead to formation of the deceptively simple layered structure of the cerebral cortex.

Addresses: *Department of Neuroscience and †Alzheimer Research Laboratory, Department of Neurology, Case Western Reserve Medical School, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA.
E-mail: kxh26@po.cwru.edu

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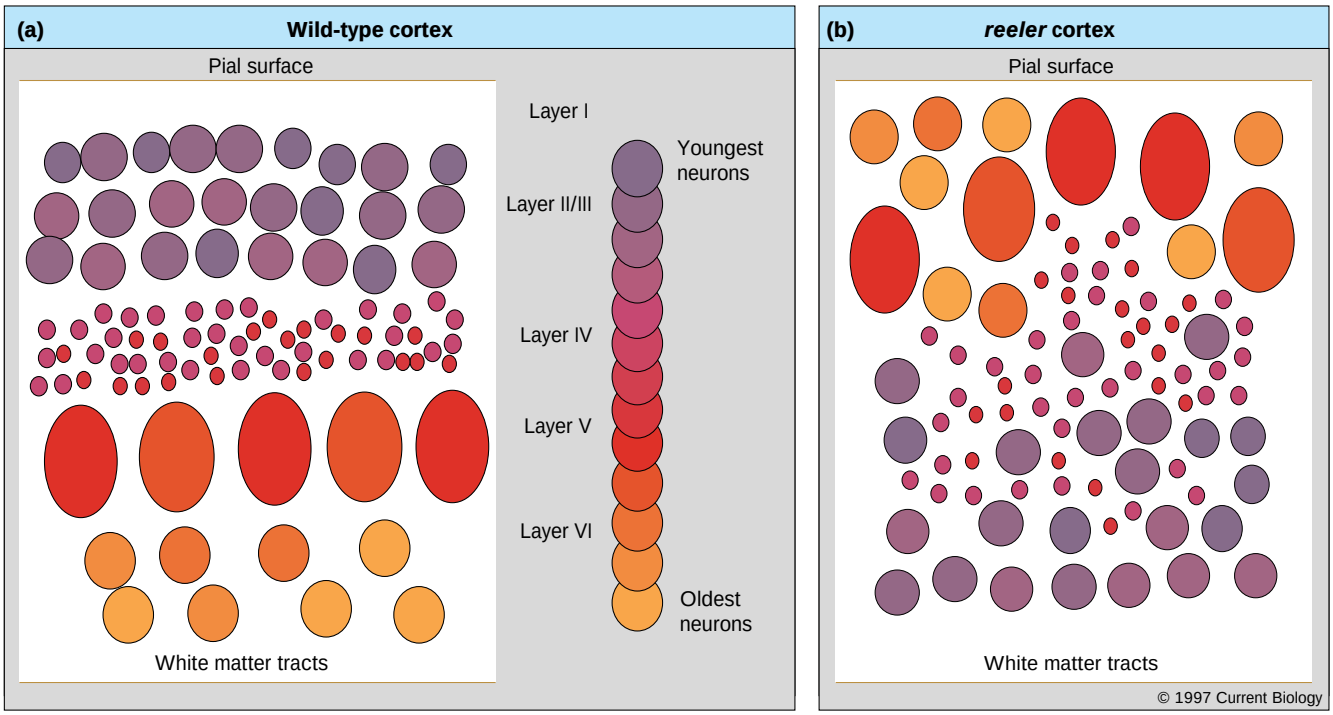
The neocortex is believed to endow mammals with the ability to perform complex tasks. The adult cortex has a simple structure, consisting of six, nearly continuous layers of cells, each with specific circuit functions. Layer I is found closest to the outer (pial) surface, while layer VI has the deepest location — just above the white matter tracts of axons that interconnect the cortex with the rest of the central nervous system (CNS) (Fig. 1a). This simple six-layer plan disguises a great deal of anatomical and physiological complexity. For example, early this century Brodmann and others subdivided the human neocortex into nearly four dozen distinct regions, based solely on the relative density and thickness of the cortical layers, and correctly predicted that each area would have different functional properties. Later, physiologists showed that the local circuitry of neocortex can be described as a series of vertically aligned columns of interconnected neurons. Neurons within a column must accurately connect, not only with other cells in their own column, but also with cells in nearby cortical columns and cells in distant parts of the brain and spinal cord.

The embryonic development of the neocortex, like the adult cortical anatomy, appears deceptively simple at first glance, but is actually a highly complex process. Virtually

Figure 1



(a) Adult wild-type cerebral cortex. The neurons are layered according to birth date. The color bar at the right is meant to indicate the relative ages among the cells. The oldest layer VI neurons are colored yellow, and the youngest layer II/III neurons are colored purple. (b) Adult *reeler* cerebral cortex. The oldest neurons (yellow/orange – normally in layer VI) tend to be located near the pial surface, while the youngest neurons (purple – normally in layer II/III) are in the lower portions of cerebral cortex. Note that, in the *reeler* cerebral cortex, the cell-sparse, marginal layer (layer I) is missing, and the cortical neurons do not stratify according to their birth date as accurately as in wild-type mice.

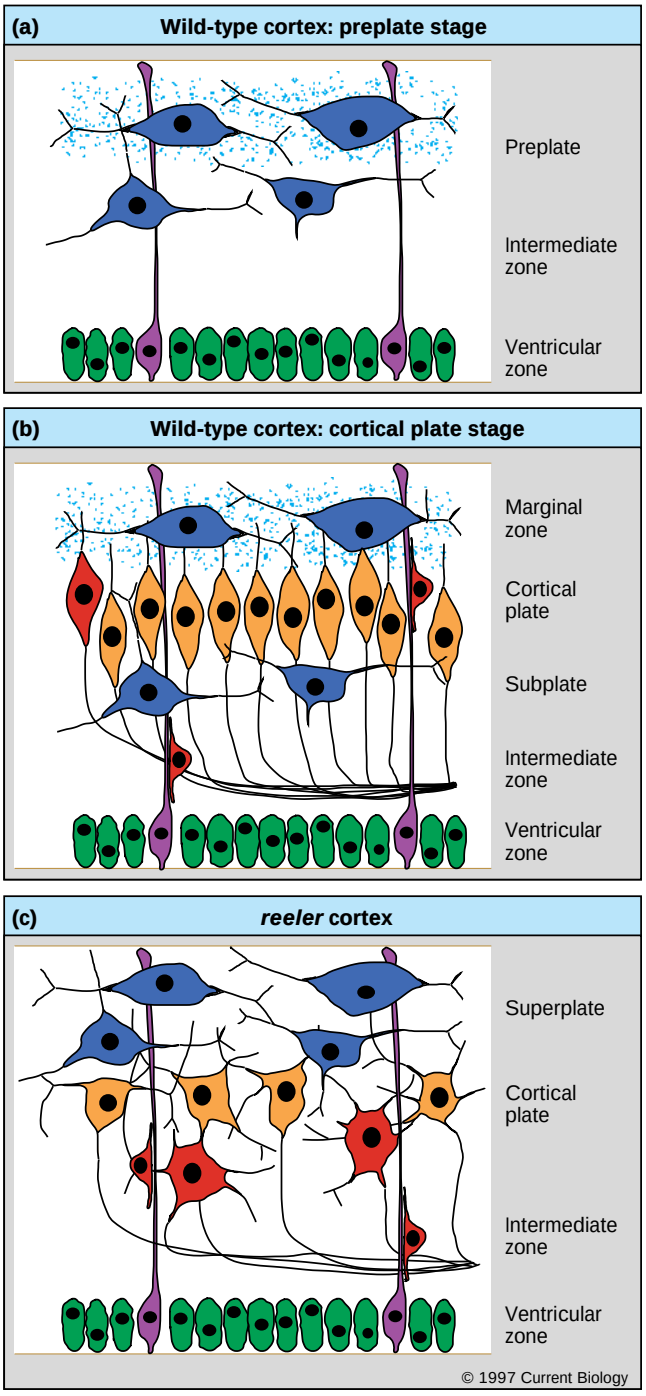
all the neurons of the CNS are generated near the lumen of the neural tube, in a region known as the ventricular zone. At specific stages of development, different populations of neurons permanently stop dividing and leave the ventricular zone. This event is such a defining characteristic of a neuron that the day of its final cell cycle is known as a neuron's birthday. After their 'birth', cortical neurons migrate centrifugally to their adult location near the pial surface. The complexity of the developmental process arises from the details of the migratory paths of the young cortical neurons.

The cortex is first visible in development as a thin layer of subpial cells known as the preplate (Fig. 2a). Next, the cells that will form the deepest cortical layer, layer VI, migrate to the preplate, which they penetrate and split into two layers: an upper marginal zone and a lower subplate [1]. As successive layers of cortical neurons are born, they migrate through the subplate and the previous neuronal layers, and stop just under the marginal zone (Fig. 2b). The end result is the stratification of cortical neurons by birthday. The oldest neurons, those of the preplate, are found in layer I (derived from the marginal zone) and deep portions of layer VI (cells derived from the subplate) of the developing cortex. The remaining cortical layers are 'inside-out' with respect to birth dates; layer II contains the youngest cells, while layer VI contains the oldest (Fig. 1a).

The anatomy and embryology of the neocortex have been studied intensively for many decades. The past several years, however, have been a particularly exciting time in this field, spurred on by molecular and genetic findings into the spatial and temporal organization of the cerebral cortex. The identification of the gene defects responsible for spontaneous neurological mouse mutants, such as *tottering* and *opisthotonos*, have alerted us to the fact that small changes in a channel or receptor protein can produce profound epileptic conditions in the adult CNS [2,3]. The discoveries of nested expression patterns of several families of transcription factors have provided insight into the mechanisms that first establish the neocortical 'field' in the embryo. It is, however, the recent studies of a naturally occurring mouse mutant, *reeler*, that have generated the most excitement.

The neurons of the *reeler* cortex are 'born' at the correct developmental times. That is, cells that will assume the morphology and functional properties of layer VI cells leave the ventricular zone and migrate to the developing cortex at the appropriate date; they are followed successively by layers V through II, but the migration patterns of these neurons are abnormal. Layer VI neurons migrate up to the preplate, but no further. As a consequence, the preplate is not split, and the subplate remains adjacent to the marginal zone (Fig. 2c). Layer V cells arrive next, but rather than migrating past the resident layer VI cells, they

Figure 2



(a) Preplate stage of cerebral cortex development. Preplate neurons are dark blue; undifferentiated ventricular zone cells are green; radial glia are purple; and Reelin, produced by Cajal-Retzius cells, is represented by light blue dots. (b) Separation of the preplate into subplate and marginal zone by the developing cortical plate in wild-type mice. Future layer VI neurons are orange; future layer V neurons are red. (c) In *reeler* mutant mice, the developing cortical plate fails to separate the preplate into two layers. Neurons that should become layer V (red) do not by pass the neurons that should become layer VI (yellow). In the *reeler* cortex, the dendritic processes are arranged in a haphazard manner, whereas the axons are mostly normal.

stop beneath them. This continues, so that the stratification of birth dates in the adult *reeler* cortex is highly abnormal (Fig. 1b). Layer I cells are still superficial, but what is normally subplate lies above layers II through VI, and these layers are inverted relative to wild-type cortex [4].

The analysis of *reeler* chimeras — mice which develop containing some wild-type and some *reeler* mutant cells — has shown that these migration defects are not intrinsic properties of the ectopic cells, but rather that cell-extrinsic factors fail to allow the otherwise competent cells to migrate properly [5]. Furthermore, the normal orientation of individual neurons is skewed in *reeler* mutants: wild-type cortical neurons have their dendritic processes oriented toward the pial surface, whereas the orientation of the apical dendrites of *reeler* neurons are haphazard [6]. Even with these profound layering and orientation abnormalities, the *reeler* cortex manages to form most cortical connections properly [7].

Several laboratories actively pursued the identification and cloning of the *reeler* gene, with the first successes reported in 1995 [8,9]. The insights gained from cloning *reeler* have significantly advanced our understanding of cortical development, but have also left us with many unanswered questions. The product of the *reeler* gene, now known as Reelin, is a 385 kDa secreted glycoprotein produced by cells in the marginal zone known as Cajal-Retzius cells [8–10]. These early-born neurons were described many years ago — they form the first synapses of the cerebral cortex and their processes are oriented horizontally rather than radially [11] — but their function as a central player in cortical development was unsuspected by most. Even their adult fate is uncertain; they may remain as layer I cells, or they may serve a transient function and then die [12,13]. Abnormalities of the Cajal-Retzius cells in the *reeler* mouse were first described by Derer over a decade ago [14], but the recent cloning of the gene has greatly increased interest in this mysterious cell type and its unusual gene product.

With the identification of the *reeler* gene, new hypotheses for how neuronal migration is regulated, and new approaches to older ideas, have been suggested. One classic hypothesis about the *reeler* defect is based on the observation that most neurons arrive in the cortical plate by migrating along the surface of a transient population of radial glial cells. In *reeler* mutants, it is argued, the arriving cells cannot penetrate the preplate and remain strongly attached to their glial guides. As a result, later-arriving neurons cannot bypass the earlier cells and, by the end of cortical development, there is an inversion of the normal layering pattern [4]. As Reelin protein (blue dots in Fig. 2a,b) is produced near the pial surface [8,9], there is no obvious direct interaction between the arrested cells and the glial guides. As the radial glia are in contact with the pial surface, however, it is possible that Reelin directs neuronal migration indirectly *via* the radial cells.

A second early hypothesis about *reeler* is that the mutation alters the adhesive properties of young cortical neurons. This idea was based on the following observation: if cortical neurons are dissociated and allowed to reaggregate in rotating culture, then cells born on different gestational days form reproducible patterns in the reaggregates, and these are altered when *reeler* cells are used. The proposed mechanism relies, therefore, not on the blocked migration of younger cells entering the cortex, but of altered adhesive properties of cells once they arrive [15].

To these older hypotheses, newer ones have been added that are based on the insights provided by the new genetic information. It has been proposed that Reelin, manufactured by the Cajal-Retzius cells, acts directly upon other neurons. Reelin may act as a repellent to subplate cells, thus explaining the splitting of the preplate that normally occurs, or as an attractant to migrating neurons, thus explaining the migration of new neuronal arrivals all the way to the marginal zone, or as both [10,16].

Other studies remind us that, even if such hypotheses are correct, radial glial cells may still play a role; recall that the radial glia of *reeler* mice are misaligned in both the cerebral and cerebellar cortices [17,18]. A recent study of migrating granule cells of the cerebellum indicates that soluble factors, produced by wild-type cells forming layer I of cerebral cortex, can cause differentiated glia to assume a radial phenotype and change the direction of migrating neurons (E. Soriano, personal communication). A 55 kDa factor, isolated from forebrain, has been shown to induce a radial-glia-like phenotype from astrocytes [19], but *reeler* astrocytes do not respond to the factor, suggesting that Reelin could play a role in the differentiation of radial glia [17]. Others have shown [20] that cell types that produce Reelin are capable of altering the direction of migrating Purkinje cells in slice preparations. These types of studies need to be extended to the cerebral cortex.

The explosion of genetic insights into the control of cortical development appears to be far from over. One example is the recently described mouse mutant known as *scrambler*, which has a nearly identical phenotype to *reeler* [21,22]. The two genes may act through independent pathways, but as Reelin is present in *scrambler* mice, it is provocative to consider that the *scrambler* gene product is involved in the downstream response of the cortical cells to the Reelin protein. One simple possibility is that *scrambler* encodes the Reelin receptor. The anticipated cloning of this new gene should facilitate a quick resolution of the possibilities. The intracellular mechanisms that are required for the migration process are also likely to be identified in the coming years. In recent months, an engineered mutation in

the gene encoding a regulatory subunit (p35) of cyclin-dependent kinase 5 (Cdk5) has been reported to produce a phenotype that is reminiscent of *reeler* [23].

The mysteries surrounding the formation of cerebral cortex are still many and varied. From the specification of the early cells, to the proper formation of the early cortical plate, to the differentiation of the circuitry and anatomy of the different cytoarchitectonic regions, much remains to be learned. The pace of discovery is quickening, aided in no small measure by the contributions of geneticists and molecular biologists. The prognosis is good for advancements in our understanding of the 'logic' underlying the layers of complexity in this important brain region.

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